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Comparison of Tests for Antibody to Hepatitis E Virus

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The results of serologic tests for hepatitis E virus have varied widely from laboratory to laboratory, making interpretation of seroepidemiologic studies difficult. The present study compares serologic results with different antigens and tests developed in two laboratories for their ability to diagnose hepatitis E and measure antibody prevalence in a high risk population in Saudi Arabia. The results confirm that tests based upon open reading frame (ORF) 3 of HEV are of limited value for seroepidemiologic studies, whereas ORF2-based antigens have broad utility and yield data that are reproducible in more than one laboratory. ***J. Med. Virol. 55:134–137, 1998***

KEY WORDS: hepatitis E; antibody; HEV genome

INTRODUCTION

The cloning and sequencing of the HEV genome resulted in the development of the first practical serologic test for anti-HEV, which utilized various expressed proteins of the virus as antigens. Prior to that time, the serology of hepatitis E was limited to immune electron microscopy, a highly specific but very cumbersome and laborious assay [Balayan et al., 1983]. However, results obtained with tests based upon recombinant HEV antigens have yielded surprising results. First, although the tests readily detected anti-HEV in convalescent sera of naturally infected humans and experimentally infected non-human primates, the relatively low proportion of the population with such antibody in regions where HEV is endemic was unexpected for an enterically-transmitted virus that is thought to be the most important cause of clinical hepatitis among adults in many countries of Asia. Thus, early tests based on small peptides detected anti-HEV prevalence rates of less than 10% [Dawson et al., 1992a] and even more sensitive tests detected such antibody in only 20–40% of adults from a region where hepatitis E is highly endemic [Arankalle et al., 1995]. Second, it was surprising to find a significant prevalence of anti-HEV among populations of industrialized countries, where hepatitis E is virtually never diagnosed. Thus, approximately 2% of normal blood donors in the United States and Europe have been found to be positive for anti-HEV [Dawson et al., 1992a].

These findings have raised questions about the sensitivity and specificity of currently available tests for anti-HEV as well as questions about how long anti-HEV can be detected after infection. For instance, Goldsmith et al. [1992] found that IgG anti-HEV could be detected for less than 6 months in about half of tested children who were convalescing from hepatitis E but Bryan et al. [1994] found all of 33 convalescent adults to be positive 20 months after infection and anti-HEV has been detected by Khuroo et al. [1993] as long as 14 years after clinical hepatitis E in India.

Many of these unexpected results and discrepancies can be ascribed to differences in the assays for anti-HEV. Specifically, the choice and size of antigen appears to make a significant difference in the results. Assays for anti-HEV based on antigenic epitopes of the ORF3 gene product detect a lower prevalence of anti-HEV, suggesting these antibodies have a shorter half-life than antibodies directed against epitopes of the gene products of ORF2. This problem is compounded by a greater genetic heterogeneity of ORF3 genes, possibly leading to serologic differences among different HEV strains, and diminished sensitivity of assays based upon only one or a limited number of genetic variants

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of the ORF3 gene product. In contrast, gene products of ORF2 are more genetically homogeneous and measure anti-HEV that remains detectable for years.

Clinical disease data suggest that hepatitis E virus (HEV) is a pathogen with a restricted geographical range. The purpose of this study was to compare hepatitis E assays developed at the National Institutes of Health (NIH) [Tsarev et al., 1993] and Genelabs (GL) [Yarbough et al., 1991, 1994] for sensitivity and specificity and to evaluate their ability to diagnose hepatitis E in patients with acute sporadic viral hepatitis who presented at one of two infectious disease hospitals in Saudi Arabia, the Al Thagher Hospital in Jeddah and the King Faisal Hospital in Makkah (Mecca) [Ghabrah et al., 1995].

MATERIALS AND METHODS

Serologic and molecular tests

Blood samples were collected from 217 consecutive patients who had a clinical diagnosis of acute viral hepatitis and were at least 13 years of age [Ghabrah et al., 1995]. Serum samples were separated and stored at −20°C upon arrival at the King Abdulaziz University Hospital Laboratory, Jeddah. All serologic tests were based on enzyme immunoassay and were performed without knowledge of the results of other serologic tests. Based on results from commercially available tests, one hundred fifty patients were diagnosed as having hepatitis A, B, C, or D [Ghabrah et al., 1995].

Tests for IgM and IgG antibody to HEV (IgM anti-HEV, IgG anti-HEV) were performed with an NIH-developed EIA utilizing, as antigen, the putative HEV capsid protein expressed from ORF2 as a processed 55 kda protein in insect cells [Tsarev et al., 1993]. Aliquots of the same sera were also tested at Genelabs, Inc. (Redwood City, CA) for IgM and IgG anti-HEV with EIAs developed at GL utilizing HEV antigens expressed as GST fusion proteins in *E. coli.* The designated 3-2 antigen comprises 42 amino acids of the carboxy end of ORF2 and contains a previously identified immunodominant epitope [Yarbough et al., 1991]. The larger ORF2 derived antigen SG3 contains the carboxy terminal 327 amino acids of ORF2, also shown to contain at least two linear epitopes [Yarbough et al., 1994]. The designated 4-2 antigen comprises 33 amino acids from the carboxy end of ORF3 and contains a previously identified immunodominant epitope [Yarbough et al., 1991]. The ORF3 protein designated ORF3-85 [Yarbough et al., 1994] contains all 123 amino acids of the ORF.

Analysis of serologic data

Tests were compared for overall concordance and for strength of agreement (Kappa statistic), where Kappa values of 0.00–0.20, 0.21–0.40, 0.41–0.60, 0.61–0.80, and 0.81–1.00 signify strengths of agreement of slight, fair, moderate, substantial, and almost perfect, respectively [Fleiss et al., 1981].

RESULTS

IgM antibody to expressed antigens of HEV ORF2

A total of 41 patients were positive for IgM anti-HEV ORF2 by one or both tests. Among the positive sera, 87.8% were from the 67 patients not diagnosed as having hepatitis A–D. Thirty-three of the 41 positive sera were positive by both tests; the Genelabs test identified five additional positives and the NIH test identified three additional positives (concordance: 96.3%; Kappa: 0.87) (Table IA). Patients who were anti-HEV ORF2 positive from the A–D group included three patients with acute hepatitis B, one with acute hepatitis D and one with markers of HCV infection of undetermined duration (classified as acute hepatitis E in the previous study [Ghabrah et al., 1995]).

High titered IgG antibody to HEV ORF2

We have demonstrated previously a positive correlation between the detection of high-titered IgG antibody to HEV ORF2 and acute hepatitis E associated with an epidemic of water-borne hepatitis E in Pakistan [Bryan et al., 1994]. In the present study, the titer of IgG anti-HEV ORF2 was determined with the NIH test. To determine if high-titered (≥1:10,000) anti-HEV ORF2 could be used in place of IgM anti-HEV ORF2 as a diagnostic test, we compared the results obtained with these two NIH tests. As seen in Table IB, the two tests were positive in 25 instances, negative in 175, and disagreed in 17, for a concordance of 92.2% and a Kappa of 0.70. High-titered IgG anti-HEV ORF2 was found in the absence of IgM antibody (by NIH test) in one case of hepatitis D and two cases of hepatitis B, as well as in three cases of hepatitis E, two of which were positive for IgM antibody and one of which was indeterminant for IgM antibody in the Genelabs test. When high-titered IgG anti-HEV ORF2 (NIH test) was compared with the Genelabs IgM test, a similar concordance (91.2%) was found (data not shown). When data on IgM and IgG anti-HEV antibodies detected in the NIH test were combined with Genelabs data on IgM anti-HEV, as many as 45 possible cases of hepatitis E were identified (Table IC).

IgG antibody to HEV ORF2

In contrast to IgM anti-HEV, which is recognized to be a marker of recent HEV infection, IgG anti-HEV is thought to be a marker of recent (if present in conjunction with IgM anti-HEV or present in high titers) or past (if low titers are found in the absence of IgM anti-HEV) infection with the hepatitis E virus. The sera from the 217 patients in the present study were tested for IgG anti-HEV by both laboratories. Ninety-seven of the 217 sera were positive for IgG anti-HEV ORF2 (Table ID). Eighty of these were detected by both tests. Fourteen additional sera were positive with the NIH test (five of these were indeterminant in the Genelabs test) and three additional sera were positive in the Genelabs test (concordance 92.2% Kappa: 0.84).

TABLE I. Concordance of Tests for Antibody to Hepatitis E Virus

A
IgM antibody to HEV ORF2

		NIH[a] Test		
IgM		+	–	Totals
only	+	33	5	38
(GL[b] Test)	–	3	176	179
	Total	36	181	217
	Concordance: 96.3%	Kappa: 0.87		

B
IgM anti-ORF2 vs. High-titered IgG anti-ORF2

		NIH IgM test		
NIH		+	–	Totals
IgG	+	25	6	31
Test	–	11	175	186
	Total	36	181	217
	Concordance: 92.2%	Kappa: 0.70		

C
Combined IgM and high-titered IgG

		NIH IgG plus IgM		
IgM		+	–	Totals
only	+	35	3	38
(GL Test)	–	7	172	179
	Total	42	175	217
	Concordance: 95.4%	Kappa: 0.85		

D
IgG antibody to HEV ORF2

		NIH Test		
		+	–	Totals
GL	+	80	3	83
Test	–	14	120	134
	Total	94	123	217
	Concordance: 92.2%	Kappa: 0.84		

E
IgM anti-ORF3 vs. IgM anti-ORF2

		GL IgM Anti-ORF3		
GL		+	–	Totals
IgM	+	5	33	38
Anti-ORF2	–	0	179	179
	Total	5	212	217
	Concordance: 84.8%	Kappa: 0.20		

F
IgG anti-ORF3 vs IgG anti-ORF2

		GL IgG Anti-ORF3		
GL		+	–	Totals
IgG	+	38	45	83
Anti-ORF2	–	2	132	134
	Total	40	177	217
	Concordance: 78.3%	Kappa: 0.49		

[a]National Institutes of Health EIA with 55 kda protein expressed in insect cells from ORF2.
[b]Genelabs EIA with proteins (smaller than 55 kda) expressed in *E. coli* from ORF2 or from ORF3.

A total of 40 sera were positive for IgG and IgM anti-HEV as measured by one or both laboratories. A single serum was positive for IgM anti-HEV but negative for IgG anti-HEV. Fifty-seven sera were positive for IgG anti-HEV in the absence of antibody to HEV of the IgM class; these were considered to be from patients who had been infected with HEV previously. One hundred nineteen sera were negative for anti-HEV. Overall, the prevalence of IgG anti-HEV (excluding the 41 patients with IgM anti-HEV) was 32.4%. There was no difference in the prevalence of IgG anti-HEV, indicative of previous infection, between those patients in the hepatitis A–D group (32.8%) and the non A–E group (31.7%).

IgM antibody to antigens expressed from HEV ORF3

Antibody to HEV has also been detected with antigens expressed from clones of ORF3 of HEV. Although the sequence of this small ORF is relatively well conserved, genetic variation has been found in the 3′ end of the ORF. Nevertheless, this region contains broadly reactive epitopes as shown by analyses of human sera, although it may have species specificity in its immunogenicity when measured with monkey sera. Therefore, this region contains B-cell epitopes that display some genotype specificity [Yarbough et al., 1991]. In contrast, epitopes in the 3′ end of ORF2 are relatively well conserved among the different genotypes of HEV [Yarbough et al., 1991]. Epitopes of proteins expressed from ORF2 and from ORF3 have been used individually and exclusively in published assays for antibody to HEV [Lok et al., 1992; Tsarev et al., 1993] and differences in detection of anti-HEV when different epitopes were employed have been noted [Lok et al., 1994]. In the present study antigens representing the two most divergent genotypes of HEV, an Asian (Burmese) and a Mexican strain were used to test the sera from the 217 hepatitis patients for anti-HEV ORF3 as part of the tests performed by Genelabs. Only five (13.5%) of the 38 sera that tested positive for IgM anti-HEV ORF2 in the Genelabs tests were also positive for IgM anti-HEV ORF3 (Table IE).

Although there was preferential reactivity to the ORF3 antigens from the Burmese HEV strain, two of the five reactive sera also recognized the ORF3 antigen expressed by the sequence of the Mexican strain. None of the sera from patients with seromarkers for acute HAV, HBV, HCV, or HDV were reactive with any of the ORF3 antigens of HEV. Thus, the detection of IgM anti-ORF3 is diagnostic of acute infection, but the restricted detection rate may reflect a more rapidly declining IgM antibody response to the ORF3 antigen than to the ORF2 antigen, resulting in a less sensitive test.

IgG antibody to HEV ORF3

A total of 38 patients tested positive for IgG anti-ORF3 using the truncated or full-length ORF3 from the Burmese HEV as antigen (Table IF). Of the 38, 36 were also reactive with the truncated ORF3 expressed from the Mexican HEV (data not shown), suggesting that genotype specificity did not play a major role in the sensitivity of this test. Two additional sera were reactive against the ORF3 of the Mexican strain but were negative for anti-HEV in all other tests and were scored as an HBV and an HCV case respectively. They probably represented false positive reactions. With

these two exceptions, all of the sera that were IgG anti-ORF3 positive were also IgG anti-ORF2 positive. The 38 reactive sera represent only 46% of the 83 sera found to be IgG anti-ORF2 positive by Genelabs. These data may suggest that IgG antibodies to ORF3 do not persist as long as antibodies to ORF2, perhaps because of different responses of the immune system to the different HEV proteins [Dawson et al., 1992b]. Antibody reactivity to ORF3 confirms an immune response to this protein although its function is unknown.

DISCUSSION

The present comparison of tests for anti-HEV is the first to make a detailed examination of the utility of the many gene products which have been utilized for assays for anti-HEV. The results clearly showed that tests for anti-HEV based upon expressed ORF2 were more sensitive for detecting anti-HEV than were tests based upon antigens derived from ORF3. This was true both for IgM anti-HEV and for IgG anti-HEV. The poorer showing of ORF3-based tests may result from a combination of a less vigorous immune response to this small protein and a shorter half-life of antibodies to ORF3.

Also, in general, larger proteins expressed from ORF2 and ORF3 were more sensitive for detecting their respective antibodies than more highly truncated proteins. This was particularly true for detecting IgG anti-ORF2: the larger NIH recombinant ORF2 protein detected 12% more positive samples than did the shorter GL protein. However, the GL protein was slightly more sensitive than the NIH protein for detecting IgM anti-ORF2. Recent studies have shown that a larger recombinant protein expressed by Genelabs from ORF2 [McAtee et al., 1996] yielded results comparable to the similarly sized NIH protein in a direct comparison [Thomas et al., submitted for publication]. However, as a caveat, full-length recombinant proteins expressed from the entire ORF2 gene have been shown to be less suitable as an antigen for detecting anti-HEV because of their insolubility [He et al., 1993].

In summary, tests based upon recombinant antigens expressed from ORF2 of HEV were more sensitive for detecting IgM and IgG anti-HEV than were tests based upon proteins derived from ORF3. The data did not support a requirement for tests that contain antigens derived both from ORF2 and ORF3 since the only samples that were positive with ORF3-based tests but negative with ORF2-based tests appeared to be false positive reactions. Finally, proteins expressed from ORF2 have been shown to measure antibodies that correlate with protection against hepatitis E [Bryan et al., 1994] whereas no such correlation has been shown for antibodies to ORF3. Another promising approach to the detection of anti-HEV, based upon the construction of a synthetic gene that expressed an artificial protein containing linear epitopes from ORFs 2 and 3 was recently reported [Favorov et al., 1996]. However, it remains uncertain that detection of antibodies to ORF3 adds anything to the serology of HEV infection.

REFERENCES

Arankalle VA, Tsarev SA, Chadha MS, Alling DW, Emerson SU, Banerjee K, Purcell RH (1995): Age-specific prevalence of antibodies to hepatitis A and E viruses in Pune, India, 1982 and 1992. Journal of Infectious Diseases 171:447–450.

Balayan MS, Andjaparidze AG, Savinskaya SS, Ketiladze ES, Braginsky DM, Savinov AP, Poleschu VF (1983): Evidence for a virus in non-A, non-B hepatitis transmitted via the fecal-oral route. Intervirology 20:23–31.

Bryan JP, Tsarev SA, Iqbal M, Ticehurst J, Emerson S, Ahmed A, Duncan J, Rafiqui AR, Malik IA, Purcell RH, Legters LJ (1994): Epidemic hepatitis E in Pakistan: patterns of serologic response and evidence that antibody to hepatitis E virus protects against disease. Journal of Infectious Diseases 170:517–521.

Dawson GJ, Chau KH, Cabal CM, Yarbough PO, Reyes GR, Mushahwar IK (1992a): Solid-phase enzyme-linked immunosorbent assay for hepatitis E virus IgG and IgM antibodies utilizing recombinant antigens and synthetic peptides. Journal of Virological Methods 38:175–186.

Dawson GJ, Mushahwar IK, Chau KH, Gitnick GL (1992b): Detection of long-lasting antibody to hepatitis E virus in a US traveller to Pakistan. Lancet 340:426–427.

Favorov MO, Khudyakov YE, Mast EE, Yashina TL, Shapiro CN, Khudyakova NS, Jue DL, Onischenko GG, Margolis HS, Fields HA (1996): IgM and IgG antibodies to hepatitis E virus (HEV) detected by an enzyme immunoassay based on an HEV-specific artificial recombinant mosaic protein. Journal of Medical Virology 50:50–58.

Fleiss JL (1981): Statistical Methods for Rates and Proportions, 2nd Edition, New York: John Wiley & Sons, pp 217–225.

Ghabrah TM, Strickland GT, Tsarev S, Yarbough P, Farci P, Engle R, Emerson S, Purcell RH (1995): Acute viral hepatitis in Saudi Arabia: seroepidemiological analysis, risk factors, clinical manifestations, and evidence for a sixth hepatitis agent. Clinical Infectious Disease 21:621–627.

Goldsmith R, Yarbough PO, Reyes GR, Fry KE, Gabor KA, Kamel M, Zakaria S, Amer S, Gaffar Y (1992): Enzyme-linked immunosorbent assay for diagnosis of acute sporadic hepatitis E in Egyptian children. Lancet 339:328–331.

He J, Tam AW, Yarbough PO, Reyes GR, Carl M (1993): Expression and diagnostic utility of hepatitis E virus putative structural proteins expressed in insect cells. Journal of Clinical Microbiology 31:2167–2173.

Khuroo MS, Kamili S, Dar MY, Moecklii R, Jameel S (1993): Hepatitis E and long-term antibody status. Lancet 341:1355.

Lok ASF, Kawn WI, Meockli R, Yarbough PO, Chan RT, Reyes GR, Lai CL, Chung HT, Lai TST (1992): Seroepidemiological survey of hepatitis E in Hong King by recombinant-based enzyme immunoassays. Lancet 340:1205–1208.

Lok ASF, Soldevila-Pico S (1994): Epidemiology and serologic diagnosis of hepatitis E. Journal of Hepatology 20:567–569.

McAtee CP, Zhang Y, Yarbough PO, Fuerst TR, Stone KL, Samander S, Williams K (1996): Purification and characterization of a recombinant hepatitis E protein vaccine candidate by liquid chromatography-mass spectrometry. Journal of Chromatography 9(6):A1280.

Tsarev SA, Tsareva TS, Emerson SU, Kapikian AZ, Ticehurst J, London W, Purcell RH (1993): ELISA for antibody to hepatitis E virus (HEV) based on complete open-reading frame-2 protein expressed in insect cells: Identification of HEV infection in primates. Journal of Infectious Diseases 168:369–378.

Yarbough PO, Tam AW, Fry KE, Krawczynski K, McCaustland KA, Bradley DW, Reyes GR (1991): Hepatitis E virus: Identification of type-common epitopes. Journal of Virology 65:5790–5797.

Yarbough PO, Tam AW, Gabor K, Garza E, Moeckli RA, Palings I, Simonsen C, Reyes GR (1994): Assay development of diagnostic tests for hepatitis E. In Nishioka K, Suzuki H, Mishiro S, Oda T (eds): "Viral Hepatitis and Liver Disease." New York: Springer-Verlag, pp 367–370.